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(54) Title: COMPOSITION FOR SIMPLE DETECTION AND/OR QUANTIFICATION OF ANTIGENIC SUBSTANCES IN BODY LIQUIDS

(57) Abstract

Diagnostic compositions comprising conjugates of ligands or antibodies or hybrids of antibodies reactive both to specific substances (e.g. antigens) on one hand and to erythrocytes on the other hand, which will induce an aggregation of erythrocytes in presence of the said specific substance in a clinically significant amount for detection and/or quantification of the said specified substance in blood, plasma, serum or other body liquids, and the use of compositions comprising mixtures of conjugates or hybrids of antibodies, reactive to different antigenic determinants on erythrocytes and/or different antigenic determinants on the said specific substance in order to increase the sensitivity of the compositions for detection and/or quantification of the said specific substance in blood, plasma, serum or other body liquids, and the use of such compositions in combinations with antibodies reactive to the said specific substances, but not to erythrocytes, in order to adjust the sensitivity of the detection and/or quantification of the said specified antigenic substance in blood, plasma, serum or other body liquids. Such antibodies, conjugates and/or hybrids may be of monoclonal or polyclonal origin. This invention may be used in veterian and human medicine for diagnosis.

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COMPOSITION FOR SIMPLE DETECTION AND/OR QUANTIFICATION OF ANTIGENIC SUBSTANCES IN BODY LIQUIDS.

BACKGROUND OF THE INVENTION

This is invention relates to compositions useful in the detection and quantification of antigenic substances in body liquids.

Antigenic substances (antigens) are molecules with structures to which specific antibodies of polyclonal and monoclonal origin can bind. General immunological literature describes the formation and production of antibodies and antibody fragments reactive to antigens of different origins.

Important antigenic substances in medical diagnosis are blood proteins, antigens associated with organism living in blood like bacterias and viruses and blood protein split product, and numerous others antigens as taught by general medical literature. Detection and/or quantification of several of these substanses are useful for the diagnosis and treatment of medical diseases. By way of example, some of these antigenic substances will be commented:

Several kinds of diseases may induce cellular necrosis. Cellular necrosis causes disintegration of the cell membrane and release of intracellular proteins out into the extracellular liquid. When myocardial infarction occurs, intracellular myoglobins leak out from the cells out into the extracellular liquid and then to the plasma of the patient. Thus increased plasma concentration of myoglobin is a valuable sign of acute myocardial necrosis. (Cullhed I & al: Acta.Med.Scand. 215, 417-425, 1984).

Formation of fibrin from fibrinogen is a part of the thrombotic process in vivo. When patients suffer from thromboembolic diseases, both thrombosis and fibrinolysis simultaneously take place. When fibrin is split by the fibrinolysis, fibrin degradation products appear in circulating blood as signs of thromboembolic processes in the body, and may be detected and quantified as antigens reactive to antibodies in immunoassays (Devine & al: Blood 68, 317-319, 1986. Elms & al.: Thrombosis & Haemostasis 50, 591-594, 1983. Mirshaj & al.: Thrombosis res. 44, 715-728, 1986. Rowbothand & al.: Thrombosis & haemostasis 57, 59-61, 1987. Heaton & al.: J.Lab. Clin.Med. 40, 588-91, 1987. Koppart & al.: Blood 68, 437-441, 1986).

The acute phase proteins constitute a group of individual proteins which share one common characteristics: Their plasma concentrations are altered when an organism, i.e. a patient, undergoes a disease involving inflammation and/or infection. Also neoplastic diseases may induce inflammatory reactions causing acute phase reactions. (Chambers RE & al: "Akute-Phase-Proteine bei entzundlichen Erkrankungen", Diagnose und Labor 36: 124-132, 1986).

C-reactive protein (CRP) was first reported in 1930 as a human serum protein that binds to the C-polysaccharide of the pneumococcus cell membrane (Tillet W.S. and Francis T: "Seriological reaction in pneumonia with a non-protein somatic fraction of pneumococcus", J.Exp. Med.52:561-571, 1930). Later it was demonstrated that C-reactive protein binds to phosphoryl choline residues and to aminoethyldihydrogen phosphate residues of this fraction, and these bindings have been utilized for the purification of C-reactive protein and serum. (Volanakais J.E. & al: J.Immunol. Methods 23, 285-295, 1978), (Potent M. & al: FEBS Lett. 88, 172-175, 1978). Later it was also discovered that CRP concentrations rise dramatically during pneumococcus infections and several forms of infectious inflammations (Abernathy TJ and Avery OP: "The occurence during acute infections of a protein not normally present in the blood", J.Exp.Med. 73: 173-182, 1941). This non-spesific response to bodily injury was classified as a part of the "acute-phase response", in which changes in the concentrations of serum proteins parallell the course of inflammation or tissue injury. Although their exact roles remain unclear, CRP and the other acute-phase proteins function as mediators, inhibitors or participants in the process of the inflammation. CRP is a trace constituent in blood, where serum levels in healthy adults normally remain below 5 mg/l. The serum concentration of CRP rises rapidly after onset of infections, inflammations and tissue injury, and falls rapidly to normal levels as healing or recovery occurs (Whicher JT, Bell AM and Southall PJ: "Inflammation: Measurement in clinical management", Diagnostic Med. 81:62, 1981). As a clinical tool CRP quantitation appears to be more useful than the standard indices of inflammation as fever, erythrocyte sedimentation rate and leucocyte count. The magnitude of the increase from normal to a acute inflammatory concentration also contributes to CRPs utility in monitoring of disease activity (Pepys MB: "C-reactive-protein 50 years on; Lancet 1:653-657, 1981).

The CRP molecule consists of 5 identical subunits held together by non-covalent bonds (Pepys MB: se above). Several methods for CRP detection and quantification are available, including immunoprecipitation (Andersen HC, and McCarty M: "Determination of C-reactive protein in blood as measure of the activity of the

disease process in acute rheumatic fever", Am.J. Med. 8: 445-445, 1950);(Wadsworth C: "A rapid spot immuno-precipitate assay method applied to quantitating C-reactive protein in pediatric sera". Scand. J. Immunol. 6: 1263-1273, 1977); latex agglutination (Singer JM, Potz CM, Pader E and Elster SK: "The latex agglutination test". Am J. Clin. Pathol 28: 611-617, 1957); radial immunodiffusion (Nilsson LA: "Comparative testing of precipitation methods for quantitation of C-reactive protein in blood serum". Acta Pathol. Microbiol. Scand. 73: 129-144, 1968);electroimmunodiffusion (Gil CW, Fisher CL, and Holleman CL: "A Rapid method for protein quantitation by electroimmunodiffusion",Clin. Chem. 17:501-504, 1971), radioimmunoassay (Claus DR, Osman AP and Gewurz H: " Radioimmunoassay of human C-reactive protein and labels in normal sera". J. Lab. Clin. Med. 87:127-128, 1976); (Shine B. DeBeer FC. and Pepys MD: "Solid phase radioimmunoassay for human C-reactive protein". Clin. Chem. Acta 117:13-23, 1981); nephelometry (Deaton CD, Maxwell KW, Smith RF and Crevelling RL: "Use of laser nephelometry in the measurement of serum proteins". Clin. Chem. 22:1465-1471, 1976); (Gil CW, Bush WS, Burleigh WM and Fisher CL: "An evaluation of a C-reactive protein assay using a rate immunonephelometric procedure". AJCP 75: 50-55, 1981); fluoroimmunoassay (Anne L, Eimstad W, Bellet N, and Fisher C: "Development of homogeneous fluorescent rate immunoassay for C-reactive protein". Clin. Chem 27: 1075, 1981); (Ullman F, Schwarzberg M and Rubenstein KE: "Fluorescent excitation transfer immunoassay: A general method for the determination of antigens", J. Bol. Chem. 251: 4172-4178, 1976); and enzyme labelled immunoassay (Gibbons I, Skiold C, Rowley GL and Ullman EF: "Homogeneous enzyme immunoassay for proteins employing beta-galactosidase". Anal. Biochem. 102: 167-170, 1981).

Serum amyloid A (SAA) consists of a single polypeptide chain of molecular weight 11 000 - 14 000. This subunit is associated with high density lipoprotein, then to a molecular weight of 85 000 - 200 000 (Anders, RF., Natvig, J., Michalsen TE & Huseby G. Isolation and characterisation of amyloid related SAA as a small molecular weight protein. Scandinavian Journal of Immunology 4, 397-640, 1977). (Rosenthal CJ. et Franklin, EC; "Variation with age and disease of an amyloid A protein related serum component". Journal of Clinical Investigation, 55, 746-753, 1975). The function of SAA is not known, but it behaves as an acute phase reactant. Its behaviour in this respect closely paralells that of CRP in terms of onset of rise and time of peak level following an acute stimulus (McAdm. P.WJ, Elin RJ. et al. "Changes in human serum amyloid A and C-reactive protein following inflammation", Journal of Clinical Investigation, 61, 390-394, 1978). SAA seems to be an apolipoprotein of a fraction of high density lipoprotein molecules.

The L1 protein is a highly immunogenic protein of about 36500 daltons that can be purified from granulocytes with good yield. It is present in the cytoplasm of virtually all resting peripheral neutrophiles and monocytes. Immunofluorescens staining demonstrate that L1 represents a secretary prduct like lysozyme (Dale, Brandtzeg, Fagerhoel and Scott: "Distribution of a new myolocytic antigen (L1) in human peripheral blood leukocytes", Am.J.Clin.Pathol,1985; 84: 24.34). Plasma levels of L1 have been determined in series of patients and compared with CRP-concentrations, blood leukocyte counts and erythrocyte sedimentation rate in blood samples from the same patients. This study demonstrates that elevated serum concentrations of L1 is part of the acute phase response (Sander, Fagerhoel, Bakken and Dale: "Plasma levels of leukocyte L1 protein in febrile conditions: Relation to aetiology, number of leukcytes in blood, blood sedimentation reaction and C-reactive protein". Scand. J. clin Lab Inves. 44, 357-362, 1984)

CRP, and SAA and L1 are presently the plasma proteins known to have the most prominent concentration differences between the normal plasma concentrations and the plasma concentrations in acute inflammatory and infectious phase reactions. Since the increase in blood concentration of CRP, SAA and L1 is a very rapid response to infections and tissue damage, their determination are especially valuable in acute medical situations. A large number of acute medical incidents take place in general medical practice far from advanced medical laboratories. Most of the known methods for the detection and/or quantification of increased CRP, SAA and L1 concentrations utilize laboratory equipment like spectrophotometers, radioactivity counters, nephelometric equipment, and a time consuming isolation of serum or plasma from whole blood is necessary. So is also the case for the latex agglutination test published for detection of pathological myoglobulin concentrations in serum or plasma after myocardial infarction. Simple qualitative latex agglutination techniques do not utilize advanced equipment, but isolation of serum and/or plasma is necessary. A fast and simple method to detect and/or quantify acute phase response of blood proteins in whole blood is thus needed.

Antibodies with specific affinity for body liquid antigens are obtained by immunization of animals with purified antigens. Monoclonal antibodies reactive to the said antigens may be obtained by fusion of spleen cells from animals immunized with antigen or fragments thereof with suitable cancer cell lines (Køhler and Milstein. "Continous cultures of fused cells secreting antibodies of predefined specificity", Nature 286, 495-497, 1975). In vivo immunization techniques may also be applied.

Several of the immunological techniques utilize modified antibodies, i.e. antibodies conjugated to enzymes or fluorescent moieties or radioactive labels. Such modifications and conjugations may be obtained by numerous different known methods, as described by OSullivan MJ, Bridges JW and Marks V: "Enzyme immunoassay: A review", Annals of Clinical Biochemistry, 1979, 16, 221-240, and other relevant immunoassay literature.

DETAILED DESCRIPTION OF THE INVENTION.

This invention relates to the use of conjugates or hybrids of different antibodies for detection and/or quantification of antigenic substances in samples of body liquids, especially in whole blood samples. Contrary to other methods using signal forming molecules like enzymes or radioactive or fluoroscent moities or advanced equipment or latex agglutination, the present invention utilizes a constituent of the blood itself to indicate the presence of elevated concentration of the relevant antigenic substances. By conjugation or hybridization of antibodies or ligands reactive to the relevant antigenic substances with antibodies reactive to erythrocyte antigens, a chemical composition is obtained which is able to aggregate erythrocytes in presence of the relevant antigenic substances.

If the relevant antigenic substances are constituted by two or more identical subunits, two or more identical conjugates or hybrides may bind to the antigenic substance and at the same time bind to the erythrocytes present in the sample or added to the sample if necessary, thus inducing an aggregation of the said erythrocytes.

If the relevant antigenic substances are not constituted by two or more identical subunits, mixtures of different antibody conjugates or hybrids may be used, characterized by at one hand all being reactive to the erythrocytes, present in the sample or if necessary added to the sample, and on the other hand reactive to different epitopes on the relevant antigenic substances.

By way of example, CRP consist of five identical subunits, thus several conjugates may bind to the same CRP molecule. In this way the conjugates and CRP build bridges between erythrocytes, forming aggregates of the said erythrocytes. Mixtures of different antibodies binding to different epitopes of e.g. L1, myoglobin, CRP or SAA may be used.

When fibrinogen and fibrin are degraded in vivo, fibrinogen degradation products of variable molecular weight are formed. Several antigens and epitopes of these degradation products have been investigated, and several of these antigenic determinants and epitopes are not expressed in fibrinogen. Conjugates of antibodies reactive to such determinants and epitopes, with antibodies, reactive to erythrocytes induce aggregation of the erythrocytes in presence of such determinants and

epitopes, thus whole blood testing for fibrinogen degradation products is made possible.

Erythrocytes of a particular species contain numerous different antigenic determinants, several of these antigens being common to all members of the species, and several antigens are common only to a limited number of the members of the species (i.e. blood group antigens) (Van Bennet: "The membrane skeleton of human erythrocytes and its implications for more complex cells", Ann. Rev. Biochem. 1985, 54: 273-304). This invention is favourably applied with conjugates or hybrids of antibodies reactive to erythrocyte antigens common to all members of the species. Peripheral erythrocyte proteins like spectrin, ankyrin and Band 3 proteins may favourably serve as determinants. This invention may also utilize antibodies reactive to all human cells, then all the cellular constituents of the blood will take part in the aggregation reaction.

Monoclonal or polyclonal antibodies to erythrocyte antigens may be obtained by immunization with erythrocyte antigens. Monoclonal antibodies reactive to all human cells have been produced by the inventor. Conjugates between antibodies or ligands reactive to the relevant antigenic substances and antibodies reactive to erythrocyte antigens or common human cell antigens may be formed by several different methods. A glutaraldehyde activation followed by conjugation is easily performed, but is not optimal from a biochemical functional point of view. Bifunctional linkers such as - but not limited to - bifunctional diimidates, bifunctional dimaleimides, bifunctional maleimido-imides, and other bifunctional linkers may be used. Also linkages between the carbohydrate moities of immunglobulins or between sulphydryl moities may be formed.

The conjugates described may be replaced by hybrids of different immunoglobulins: Immunoglobulins consist of different polypeptides which are held together by disulfide linkages. These disulfide linkages may undergo reductive cleavage and the fragments formed may be separated under acid conditions. By neutralization, such fragments will reunite. If the fragments formed from antibodies reactive to erythrocyte antigens are mixed and then allowed to reunite with fragments formed from antibodies reactive to the relevant antigenic substances, hybrids of antibodies which are reactive both to the relevant antigenic substances and reactive to erythrocytes may be formed.

In a special embodiment for the detection and/or quantification of C-reactive antibody, the protein conjugates described may be replaced by conjugates between antibodies, reactive to erythrocytes or to all blood cells, and ligands containing phosphorylcholine residues or aminoethyldihydrogen phosphate residues, because these ligand residues bind to C-reactive protein in presence of Ca (II).

Immunological purification of the hybrids or the conjugates, on one hand being reactive to erythrocytes and on the other hand being reactive to the relevant antigenic substance, may be purified by affinity chromatography.

When such conjugates or hybrids have been isolated, they may be kept in solution, if necessary with preservatives, or lyophilized, for later use. In the actual clinical situation, these conjugates or hybrids can be combined with whole blood from the patient, most practically with capillary blood obtained by microsampling. The solution of conjugates or hybrids, or the redissolved lyophilized dry solid obtained, may contain anticoagulants prohibiting coagulation of the blood added. After a short incubation time, aggregation of the erythrocytes is observed if the relevant antigen is present in an appropriate concentration in the blood added. The sensitivity of the aggregation reaction is adjusted by adjustment of the amount of conjugate or hybrid present.

Aggregation of the blood cells may be observed visually in tubes, on slides from glass or plastics or by other techniques found practical. A more precise quantification of the relevant antigenic substance present may be obtained by particle counting, i.e. by counting the number of aggregated and/or non-aggregated erythrocytes, turbidimetric quantification, optotermic spectroscopy, thin layer chromatographic separation of aggregates from free non-aggregated erythrocytes, and other measuring techniques.

Antibodies to different antigenic determinants, epitopes, on relevant antigenic substances and to different antigenic determinants on erythrocytes are available. By using antibodies, hybrids or conjugates reactive to on one hand erythrocytes and on the other hand different epitopes on the relevant antigenic substance , may be formed. These type of conjugates or hybrids may be used in combination to strengthen the aggregation reactions and to increase the sensitivity of the test: In this way more than one single (type of) conjugate or hybrid may serve as linker between the relevant antigenic substance and the erythrocytes, thus facilitating the aggregation reaction. Such compositions comprising mixtures of different conjugates

or hybrids may favourably be in the very same buffer solution or in the same lyophylized solid, thus a simple mixing of the claimed composition and the blood from the invidial to be tested may be the only technical handling necessary, optionally followed by instrumental handling for quantitative measurement of the aggregation reaction.

If only plasma or serum is available from the individual to be tested, a suitable amount of erythrocytes from any individual may be added to form the necessary aggregates.
Tests may also, by additon of erythrocytes, be performed on samples of urin or cerebrospinal fluid.

Such compositions for detection and/or quantification of antigenic substances present in a sample of body liquid will have a certain measuring range. To obtain aggregation reactions at the clinically significant concentrations of the relevant antigenic substance, the amount of ligand or immunoglobulin conjugate or hybrid present must be adjusted. The sensitivity for antigenic substance detection and/or quantification at the optimized conjugate/hybrid concentration may be adjusted or decreased by addition of antibodies reactive to the relevant antigen, which have not been conjugated or hybridized with antibodies reactive to erythrocytes. These adjustments must be performed according to the actual clinical application, i.e. how much blood should be combined with the composition, and how sensitive to elevated antigenic substance concentrations the test should be.

Both antibodies of monoclonal and polyclonal origin may be used in the present invention. However, monoclonal antibodies are easier to standardize, conjugate and hybridize and will give higher yields after immunological purification. If available, non-immunoglobulin ligands that bind to the relevant antigenic substance may replace the antibodies. E.g. phosphorylcholine residues or aminoethyldihydrogen phosphate residues may replace anti-CRP-antibodies, since such ligands binds to CRP.

The compositions according to this invention may be formed to aggregate eryth-rocytes of the human species or of other species in presence of the relevant antigenic substance in a clinically significant concentrations in samples of body liquids.

EXAMPLES:

1. Mouse IgG monoclonal antihuman C-reactive antibodies were formed by conventional hybridoma technique and isolated from mouse ascites. Similarly mouse monoclonal IgG reactive to human erythrocyte membranes was produced by conventional hybridoma technique. Both the anti CRP antibodies and the antierythrocyte antibodies were dissolved in sodium phosphate buffer at neutral pH. N-succinimidyl 3 - (2-pyridyldithio) propionate (abbreviated SPDP) was dissolved in ethanol to 20 mM concentration. The SPDP solution was added to both immunoglobulins solutions in 3-4 molar excess to the immunoglobulins. The solution was left to react for 30 minutes at 23 °C with occasional stirring. Excess reagent and low molecular weight reaction products were removed by gelfiltration with Sephadex G-25 gel chromatography. By the same gelchromatography, the antierythrocyte antibodies were transferred to 0.1 M sodium acetate buffer pH 4.5 containing 0.1 M NaCl and dithiothreitol were added to 50 mM final concentration. The reduction by dithiothreitol was allowed to proceed for 20 minutes at room temperature. The excess reducing agent and the pyridine-2-thione formed were removed by gelfiltration with Sephadex G-25 and sodium phosphate buffer (0.1 M, pH 7.5 containing 0.1 M NaCl. This reduction was followed by mixing of the two immunoglobulin solutions, whereby conjugates between the anti-CRP-antibodies and the anti-erythrocyte-antibodies were formed. The reactive non-conjugated immunoglobulins and the pyridine-2-thione formed were subsequently removed by gelchromatography. Immunological purification of conjugates reactive on one hand to C-reactive protein and on the other hand to erythrocytes was performed by affinity chromatography.

2. Monoclonal mouse IgG reactive to all human cells (pan-human-antibodies) was produced and purified by conventional hybridoma technique from a cell line available in the laboratory. P-aminophenylphosphoryl choline acid was conjugated to the antibodies in a one step reaction by means of bis-(sulfosuccinimidyl)-suberate, where the p-aminophenylphosphoryl choline acid was present in 15 molar excess to the antibodies, and bis-(sulfosuccinimidyl)suberat was present in 20 fold molar excess to the antibodies. The conjugate was purified by gel chromatography. Immunological purification of conjugates reactive on one hand to C-reactive protein and on the other hand to erythrocytes was performed by affinity chromatography.

3. Equivalent to Example 1, though antibodies reactive to CRP was replaced by antibodies reactive to myoglobin.

4. The conjugate described in Example 1 was demonstrated to have the ability to aggregate the erythrocytes in presence of human C-reactive protein. By addition of non conjugated antibodies to CRP it was demonstrated that the conjugate described in example 1 required higher concentration of CRP to be present when non-conjugated antibodies reactive to CRP had been added, demonstrating that non-conjugated antibodies reactive to the same epitopes on CRP as the conjugate described in example 1 could be used to reduce the sensitivity of the aggregation reaction. Thus a test system could be adjusted to induce aggregation of erythrocytes when a clinically significant concentration of CRP is present.

5. Monoclonal antibodies reactive to human fibrin degradation products - but not to fibrinogen - and mouse monoclonal anti-human erythrocyte antibodies were produced by conventional hybridoma technique. The anti-erythrocyte monoclonal antibodies and the anti-human fibrin degradation products monoclonal antibodies were conjugated by SPDP as described in example 1. The conjugates were isolated, and the conjugates reactive on one hand to the erythrocytes and on the other hand to fibrin degradation products were purified with affinity chromatography techniques.

6. By the use of the compositions examplified, erythrocytes were made to aggregate in presence of CRP or myoglobin or fibrin degradation products. The aggregation could be visually observed on transparent slides and in tubes. Further more, it was demonstrated that the aggregation reaction reduced the absorption of light passing through a cuvette where the erythrocytes were suspended. Furthermore, it was demonstrated that, while erythrocytes migrated in chromatography paper, aggregates were withheld to a reduced travelling distance.

CLAIMS

What is claimed:

1. Diagnostic compositions characterized by comprising conjugates between:

a) Immunoglobulins or immunoreactive fragments of immunoglobulins with affinity for human erythrocytes or for human blood cells including erythrocytes, and

b) immunoglobulins or immunoreactive fragments thereof or ligands that bind to a specific substance (e.g. antigen)

and will cause an aggregation of human erythrocytes or human blood cells including erythrocytes in presence of the said specific substance, for detection and/or quantification of the said specific substance in samples of blood, plasma, cerebrospinal fluid, urine or other body liquids.

2. Diagnostic compositions according to claim 1, where the immunoglobulins or immunoreactive fragments thereof or ligands that bind the to a specific substance are characterized by being able to reacting with human C-reactive protein, fibrinogen degradation products, myoglobin, L1-protein or serum amyloid A protein.

3. Diagnostic compositions according to any of claim 1 or 2, where different antibodies have been hybridized instead of being conjugated to obtain the aggregation inducing ability.

4. Diagnostic compositions according to any of claim 1, 2, or 3, comprising mixtures of different conjugates and/or different hybrids.

5. Diagnostic compositions according to any of the claims 1 - 4, where the ligands that bind to C-reactive protein are ligands containing phosphoryl choline residues or aminoethyl dihydrogen phosphate residues.

6. Diagnostic compositions according to any of claim 1-5 that in addition comprise antibodies or ligands reactive to the specific substance, but that has not been conjugated to antibodies reactive to erythrocytes or human blood cells including erythrocytes.

7. Diagnostic compositions according to any of claim 1-6, where the antibodies, antibody conjugates, antibody hybrids or fragments thereof are reactive to antigenic substances from other species than the human species.

8. The use of compositions according to any of the claims 1- 7 for detection and/or quantification of specific antigens in samples of body liquids, including C-reactive protein, myoglobin, serum amyloid A-protein, L1 protein and fibrinogen degradation products and other antigens of diagnostic interest.

9. The use, according to claim 8, of compositions according to any of the claims 1-7, characterized by measurements of the aggregation reaction by means of turbidimetry of suspension of the erythrocytes, the heat generated by optothermal spectroscopy, the chromatographic characteristics in thin layer or paper chromatography or the visual observation on slides or tubes.

10. Diagnostic kits of reagents and equipment comprising compositions according to any of the claims 1-7 for the use according to claim 8 or 9.

INTERNATIONAL SEARCH REPORT

International Application No PCT/N089/00021

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC [4]

G 01 N 33/563, 33/536, 33/577

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC 4	G 01 N
US Cl	436:512, 536, 539

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

SE, NO, DK, FI classes as above.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	MEDLINE (NLM Database) Accession number 05223293 Sabouraudia 1984, 22 (1) p. 73-7, (de Camargo Z.P. et al.) "Titration of antibodies to Paracocoidioides brasiliensis by erythro-immunoassay (EIA)".	1-3, 7-10
X	MEDLINE (NLM Database) Accession number 06193671 J Immunol Methods Apr 2 1987; 98 (1) p. 83-9, (Germani Y. et al.) "Antibody chimera technique applied to the detection of Escherichia".	1, 8, 10
X	MEDLINE (NLM Database) Accession number 05946792 Eur J Immunol Jun 1986, 16 (6) p. 679-83 (Lansdorp P.M. et al.) "Cyclic tetramolecular complexes of monoclonal antibodies: a new type of cross--linking reagent".	1, 7, 10
X	MEDLINE (NLM Database) Accession number 05505845 Biomed Biochem Acta 1984, 43 (10) p. 1191-8, (Porstmann T. et al.) "Application of antibody chimera in enzyme and erythro immunoassay".	1, 3, 7

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1989-06-09	1989 -06- 0 9
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Carl Olof Gustafsson

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Proc. Natl. Acad. Sci. USA, Vol. 83, October 1986, p. 7989-7993, (M.R. Suresh et al.) "Advantages of bispecific hybridomas in one-step immunocytochemistry and immunoassays",	1
Y	see in particular pages 7992-93	9
A	GB, A, 2 167 086 (HYBRITECH INCORPORATED) 21 May 1986	1-10
Y	Patent Abstract of Japan, Vol. 8, No 141 (P-283) abstract of JP 59-40166, publ. 1984-03-05	4